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Synthesis of 6β-N(5)-Methyl-5,6,7,8-tetrahydro-L-biopterin*

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Summary

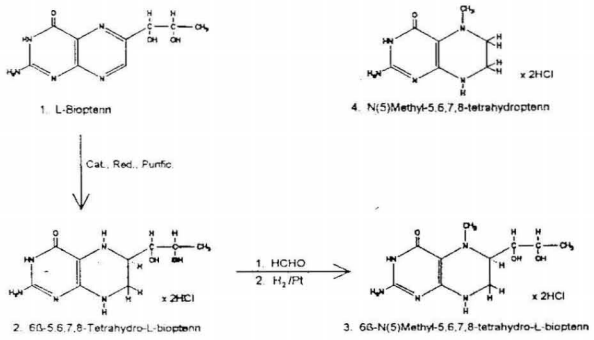
6β-N(5)-methyl-5,6,7,8-tetrahydro-L-biopterin·2 HCl, a new derivate of biological interest, was synthesized from 6β-5,6,7,8-tetrahydro-L-biopterin·2 HCl and characterized. The latter compound has been used therapeutically in atypical phenylketonuria for many years. The catalytic reductive methylation with PtO_2/H_2 and formaldehyde allowed selective methylation at the N(5) position. The chemical properties and structures of the new compound are compared with N(5)-methyl-tetrahydropterin and N(5)-methyl-tetrahydrofolate.

Key words: 6β-N(5)-methyl-5,6,7,8-tetrahydro-L-biopterin, Catalytic reductive methylation, 6β-5,6,7,8-tetrahydro-L-biopterin, 6β-5,6,7,8-tetrahydro-L-biopterin, L-biopterin, N(5)-methyl-5,6,7,8-tetrahydro-pterin, Hyperphenylalaninemia, Phenylketonuria.

Introduction

6β-5,6,7,8-Tetrahydro-L-biopterin (BH_4, **2**) is the cofactor of aromatic amino acid monooxigenases, enzymes that control the biosynthesis of several neurotransmitters (1, 2). Patients with BH_4 deficiency may suffer from mental retardation and other symptoms. Treatment of these patients requires high doses of this cofactor (10-20 mg/kg, per os), possibly due to its limited penetration through the blood-brain barrier.

We synthesized various BH_4 analoges with the aim to obtain compounds with better penetration properties that could be applied in lower concentrations. In the course of these studies, we synthesized for the first time 6β-N(5)-methyl-5,6,7,8-tetrahydro-L-biopterin (**3**) (Scheme 1). Its synthesis and chemical properties are described in this paper. In future experiments we will now be able to test the *in vivo* cofactor activities and brain penetration abilities of **3**.



The regiospecific L-biopterin synthesis from L-rhamnose or 5-deoxy-L-arabinose with 4-hydroxy-2,5,6-triaminopyrimidine was reported earlier (3, 4). For the nomenclature α, β of the two C(6)-diastereoisomers of 5,6,7,8-tetrahydro-L-biopterin see Ref. (5). The natural form is 6β-(earlier called 6R-); according to IUPAC rules the configuation is 1'R, 2'S, 6R. The coenzyme function of BH_4 is related to the redox system: 6β-5,6,7,8-tetrahydro-quinonoid-6,7-dihydro[8H]-L-biopterin. The title compound **3** may be

*This work is dedicated to Prof. Hans-Christoph Curtius on the occasion of his 70th birthday.

as interesting as the homologous N(5)-methyl-tetrahydrofolate (6).

The synthesis of 6β-N(5)-methyl-tetrahydro-L-biopterin is illustrated in Scheme 1. L-Biopterin is converted to BH_4 (**2**) by catalytic hydrogenation in hydrochloric acid solution. Pure 6β-diastereoisomer was used for further synthesis. The catalytic reductive methylation at N(5), as the strongest basic NH-group of the molecule, is described in detail (modified general method) in the Experimental section. In an earlier publication by Matsuura and Sugimoto (7) the methylation method developed by Rylander (8) was applied to tetrahydropterin (**4**) and its 6-alkyl derivatives. In a later electrochemical oxidation study 7,8-dihydropterin was formed from **4** under release of the 5-methyl group (9).

Results and Discussion

N(5)-methyl-tetrahydro-L-biopterin (**3**) is more stable to oxidation than the starting material **2** and has different physicochemical properties, i.e., reversed optical rotation. The appearance of the blue fluorescence on the TLC-plate after several hours of exposure to air requires the release of the methyl group. The UV-spectrum (see Table 1) is similar to that of N(5)-methyl-tetrahydropterin (7) and also pH-dependent (amphoteric character), with pK_a determination possibilities. Chirale HPLC control analysis as for the diastereoisomers 6α/6β-separation of tetrahydro-L-biopterin has so far not been done.

The spectral data of the new compound are shown in Figures 1 and 2 (^{1}HNMR), and in Table 1 and Figure 3 (UV), followed by mass spectrometry data.

^{1}H-NMR

(0.1 N DCl/D_2O) (Fig. 1): 4.05-3.99 d_d, J=5, H-C (7 eq); 3.98-3.92 m, H-C (6) and H-C (2′=; 3.84-3.78 d_d, J=5, H-C (7 ax); 3.43 broad s, H-C (1′); 3.22 s, CH_3-N (5); 1.31 d, J=2, CH_3-C (2′). After irradiation at 3.43 (double resonance) (Fig. 2), the peak 3.43 disappears and the m changes in the new signals at 3.98-3.96 d, J=2, H-C (2′) and 3.92 t, H-C (6). For the assignment of the ^{1}H-NMR peaks of **3** we use the results we have already obtained with other tetrahydropterin derivatives (10-12).

GC/MS Analysis of 6β-N(5)-methyl-tetrahydro-L-biopterin·2 HCl

The sample was derivatized by a mixture of BSTFA/acetonitrile (1:3, v/v) for 60 min at 100℃ to the TMS ethers, as described (13). *GC*: Carlo Erba, 10 m fused silica SE-54 column, 0.8 bar He; injector 320℃, temperature program 80℃/min to 200 ℃ within 12 min, then increased to 250℃ at a rate

Table 1. UV spectrum of 6β-N(5)methyl-5,6,7,8-tetrahydro-L-biopterin·2 HCl.

Solution	pH	λ_1 nm (ε_1=molar extinction)	λ_2 nm (ε_2=molar extinction)	Ion state
1 N HCl	0	212 (10315)	262 (15025)	Di-cation
0.1 N HCl	1	220 (16500)	264 (12700)	
1/15 M KH_2PO_4	3.5*	222 (17815)	264 (11500)	(mono-) cation
H_2O	4	222 (17770)	264 (10055)	
1/15 M KH_2PO_4/ Na_2HPO_4 (Socrensen)	8.2	220 (17262)	286 (11129)	zwitterion (neutral molecule)
0.1 N NaOH	13	222 (12540)	278 (9035)	anion (stability?)

*pH adjusted with 1 N HCl. The largest pH-dependent bathochromic shift (see λ_2) occurs between cation ion state and neutral molecule. The max. molecular extinction (MW: 328.20) is in the same pH range (see ε_1) in accordance with Fig. 3.

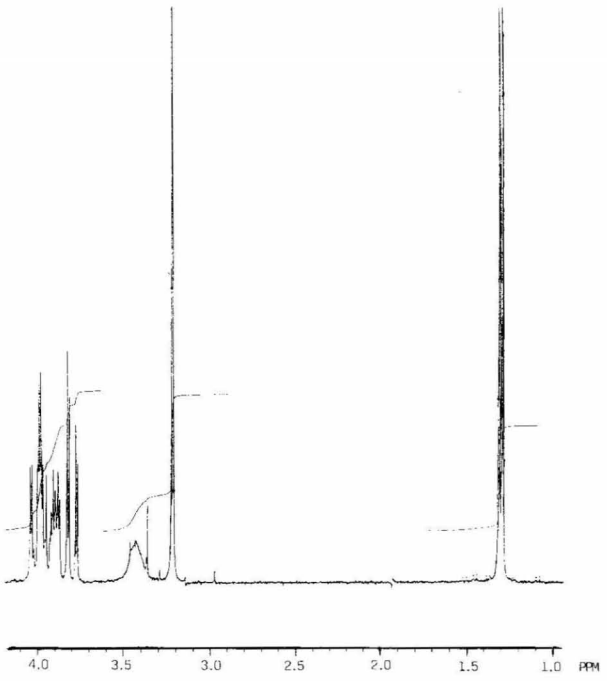


Figure 1. ^{1}H-NMR spectrum of 6β-N(5)methyl-5,6,7,8-tetrahydro-L-biopterin·2 HCl in 0.1 DCl/D_2O.

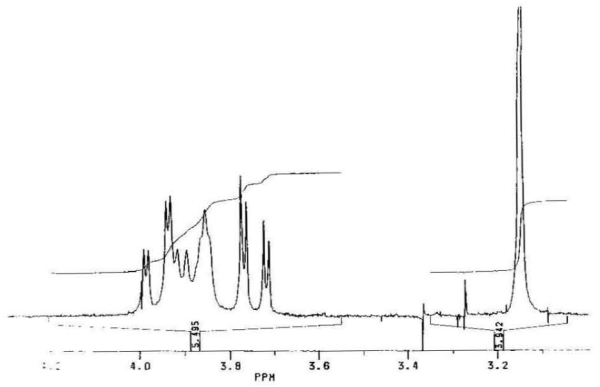


Figure 2. ^{1}H-NMR spectrum of **3**, irradiated at H-C (1'), 3.43 ppm).

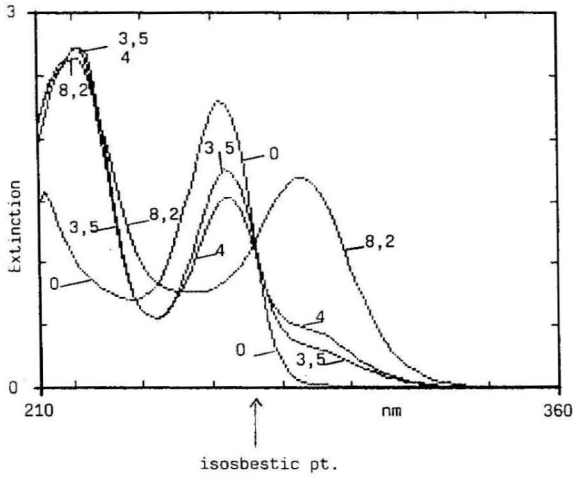


Figure 3. UV spectrum of 6β-N(5)-methyl-5,6,7,8-tetrahydro-L-biopterin 2 HCl. Curve number represents pH of solution (C = 50 mg **3**/L).

of 4°C/min. *MS*: VG-7070F, EI^+ with 40 EV, ion source temperature 220°C, emission current 50 μA, scan range m/e 100-750 at a rate of 1.5 sec. *Result*: The title compound showed a weak signal for the molecular ion with four trimethylsilyl groups (m/e 543, 0.5% relative intensity), as common for pteridines; the spectrum showed peaks for silyl derivatives with fewer (three) TMS groups (m/e 471); the base peak (m/e 324) is formed by loss of the entire side chain at C-6 and two remaining TMS groups.

TLC

Silica gel $K60F_{254}$ plates (Merck); solvent: isopropanol-H_3BO_3 (0.5 M, in H_2O)-H_2O (4:1:0.5). **3**: R_F 0.59 (fluorescence appeared only after 24 h exposure to air); **4**: R_F 0.34.

HVE

Electrophoresis on paper (Schleicher-Schüll No. 2043B) with buffer, pH 1.9: acetic acid-formic acid-water (120:26:850 v/v). **3**: R_x 203; **4**: R_x 248 in relation to L-biopterin: R = 100.

Experimental Part

Apparatus

The optical rotation was measured with a Perkin Elmer Polarimeter Type 241; the ^{1}HNMR spectrum was recorded on a Brucker 300 MHz NMR spectrometer, and the UV spectrum was registered with a Diode Array HP8451A spectrophotometer.

Chemicals

Formaldehyde, 36% in H_2O, stabilized with 10% methanol, platinum IV-oxide (Adams catalyst), methanol puriss. and diethylether abs. (Fluka, CH-9470 Buchs, Switzerland), 6-β-5,6,7,8-tetrahydro-L-biopterin·2 HCl (Schircks Laboratories, CH-8645 Jona, Switzerland).

6β-N(5)-Methyl-5,6,7,8-tetrahydro-L-biopterin·2 HCl (3): In a hydrogenation apparatus (round bottom 3-neck flask, 250 ml) with magnetic stirrer, nitrogen flow device, and H_2-balloon (connected with a hydrogen bomb for temporary reloading), acetic acid (3 ml, 2 N) and methanol (40 ml) were added to the PtO_2-catalyst (90 mg maximum). After N_2 flow the catalyst was prehydrogenated for approx. 20 min under H_2-balloon pressure, stirring, and cooling (ice water bath). 6β-5,6,7,8-Tetrahydro-L-biopterin·2 HCl (315 mg, 1.0 mmol) was added (solid) under N_2-flow in the reaction bulb. A mixture of acetic acid (3 ml, 2 N) and methanol (20 ml), followed by formaldehyde 36% (0.48 ml, 6.2 mmol) was added and the hydrogenation (i.e. the reductive methylation) was started under stirring and H_2-balloon pressure for 2 h at 15°C. After N_2-flow a second portion of formaldehyde 36% (0.3 ml, 3.8 mmol) was added and again hydrogenated for 2 h (ca. 15°C) under stirring. The catalyst was filtered off, protected under N_2-flow over a glass filter D4, covered with Celite 535 (argon protection of the filter to avoid self ignition of catalyst), and rinsed with methanol 90% (15 ml). The filtrate was evaporated (Rotavapor) to ca. 10 ml, then HCl (100 μl, 6 N) and ethanol abs. (20 ml) were added and the filtrate was reconcentrated to 5 ml. Ethanol (abs., 30 ml) was added again and evapora-

ted to a slurry (ca. 5 ml). On addition of ether (abs., 30 ml) under ice bath cooling, the white dihydrochloride was left to cristallize overnight at 4°C. The product was collected (D4 glass filter), washed with a few ml of a mixture of ethanol-ether (abs., 1:1), followed by ether and dried in a vacuum desiccator over P_2O_5 and NaOH pellets separately at room temperature: 0.31 g dihydrochloride (**3**), yield ca. 95 %.

For recristallization the dihydrochloride (0.3 g) was dissolved in warm methanol (20 ml) under addition of HCl (50 µl, 6 N) and water (600 µl). After addition of carbon (10 mg) the solution was filtered (glass filter D4, covered with Celite 535), and the filtrate was chilled. HCl (800 µl, 6 N) and, gradually, diethylether (25 ml) were added under cristallization of the product. After 2 h in the ice bath the cristals were filtered, washed with up to 10 ml cold ether /methanol (2:1), then washed with ether and dried in a vacuum desiccator; yield: 0.25 g 6β-N(5)-methyl-5,6,7,8-tetrahydro-L-biopterin·2 HCl.

Analysis: calc. for $C_{10}H_{17}N_5O_3$ 2 HCl (328.20): C 36.60, H 5.84, Cl 21.60, N 21.34%; found C 36.40, H 5.68, Cl 21.32, N 21.08%.

Specific optical rotation: $[\alpha]^{21°}_{D(589\,nm)}$ +44.8°(C=1.056; 0.1 N HCl). (In comparison 6β-tetrahydro-L-biopterin: $[\alpha]^{21}_{D}$-65.2°(C=1.135; 0.1 N HCl).

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References

1. Scriver CR, Kaufman S, Woo SLC. The hyperphenylalaninemias. In: Scriver CW, ed. The metabolic basis of inherited disease I. 6th ed. New York: McGraw Hill, 1989; 495-562.
2. Curtius H-Ch, Blau N, Kuster T. Pterins. In: Hommes FA, ed. Techniques in diagnostic human biochemical genetics, a laboratory manual. New York: Wiley-Liss, 1991; 377-96.
3. Schircks B, Viscontini M. A new regiospecific synthesis of L-biopterin. Helv. Chim. Acta 1985; 68: 1639-42.
4. Taylor EC, Jacobi R. A total synthesis of L-erythrobiopterin. J. Am. Chem. Soc. 1976; 98: 2301-06.
5. Viscontini M. Designation and representation of tetrahydropterins. In: Levine RA, Milstien S, Kuhn D, Curtius H-Ch, eds. Pteridines and biogenic amines in neuropsychiatry, pediatrics, and immunology. Grosse Pointe: lakeshore Publishing Co., 1989; 33-50.
6. Benkovic SJ. On the mechanism of action of folate and biopterin requiring enzymes. Ann. Rev. Biochem. 1980; 49: 227-5.
7. Matsuura S, Sugimoto T. Heterocyclic chemistry, Part VIII: Synthesis of 5-alkyl-tetrahydropteridines. Res. Bull. Dept. Gen. Educ. Nagoya Univ. 1969; 13: 9-17.
8. Rylander PN. Catalytic hydrogenations over platinum metals. New York: Academic Press, 1967; 291-3.
9. Karber LG, Dryhurst G. Electrochemical oxidation of 5-methyl-5,6,7,8-tetrahydropterin. J. Electroanal. Chem. 1984; 160: 141-57.
10. Weber R, Viscontini M. Zur Konformation von 6-Methyl- und 5,6-Dimethyl-5,6,7,8-tetrahydropterin. Helv. Chim Acta 1975; 58: 1772-1780.
11. Weber R, Viscontini M. Zur Konfiguration and Konformation von 6,7-Dimethyl- und 5,6,7-Trimethyl-5,6,7,8-tetrahydropterin. Helv. Chim. Acta 1976; 59: 2379-2382.
12. Weber R, Viscontini M. Konformationsanalyse von acyliertem 5,6,7,8-Tetrahydropterin. Helv. Chim. Acta 1977; 60: 161-165.
13. Kuster T, Niederwieser A. Gas chromatography-mass spectrometry of trimethylsilylpteridines. J. Chromatogr. 1983; 278: 245-54.